VI. Specification Amendments under 37 C.F.R. § 1.121

1) In the "Summary of the Invention" section on page 2 of the instant specification, please replace the paragraph that starts with the following sentence, "The present invention further relates to method of treating an individual having blood coagulation defect..." with the following amended paragraph:

> The present invention further relates to method of treating an individual having blood coagulation defect (*e.g.*, hemophilia A, hemophilia B), comprising administering to the individual an effective amount of a DNA vector expressing modified Factor VII (FVII), wherein the modified Factor VII leads to generation of Factor VIIa *in vivo*. In one embodiment, the modified Factor VII comprises an amino acid sequence which codes for a signal for precursor cleavage by the protease furin at the activation cleavage site of the modified Factor VII. For example, the amino acid signal in the modified FVII can comprise an Arg149-X150-Lys151-Arg152 (SEQ ID NO. 17) signal sequence or an Arg149-X150-Arg1[[4]]51-Arg152 (SEQ ID NO. 18) signal sequence, such as Arg149-GLN150-Lys151-Arg152 (SEQ ID NO. 10). In another embodiment, the DNA vector encoding modified Factor VII is administered as a combination of two compositions wherein the first composition comprises the light chain (from about amino acid 1 to amino acid 152) of human Factor VII and the second composition comprises the heavy chain from about (amino acid 153 to about amino acid 406) of human Factor VII and (operably linked to) a leader sequence (*e.g.*, derived from a cytokine or a clotting factor). The DNA encoding modified Factor VII of the present invention can be administered as any gene transfer vector, such as viral vectors, including adenovirus, AAV, retrovirus and lentivirus, as well as plasmid DNA with or without a suitable lipid or polymer carriers, and is administered under conditions in which the nucleic acid is expressed *in vivo*. Alternatively, the DNA encoding modified FVII can be administered as naked DNA or in association with an amphiphilic compound, such as lipids or compounds, or with another suitable carrier.

2) In the "Brief Description of the Figures" section, on page 5 of the instant specification, please replace the paragraph that starts with the following sentence, "Figure 4 illustrates clotting time of 293 cells..." with the following amended paragraph:

> Figures 4A and 4B illustrates clotting time of 293 cells [Fig. 4A] and Hep3B cells [Fig. 4B] untransfected, and transfected with FVII and FVIIa.

3) In the "Detailed Description of the Invention" section on page 8 of the instant specification, please replace the paragraph that starts with the following sentence, "In a particular embodiment, the modified FVII comprises an amino acid sequence which codes for a signal for precursor cleavage by..." with the following amended paragraph:

> In a particular embodiment, the modified FVII comprises an amino acid sequence which codes for a signal for precursor cleavage by furin. Furin is a ubiquitously expressed protease that resides in the trans-golgi and processes protein precursors before their secretion. Furin cleaves at the COOH-terminus of its consensus recognition sequence, Arg-X-Lys-Arg (SEQ ID NO. 17) or Arg-X-Arg-Arg (SEQ ID NO. 18), and to a lesser extent, Arg-X-X-Arg (SEQ ID NO. 8). The amino acid (aa) sequence at position 149-152 of human FVII is Pro-Gln-Gly-Arg (SEQ ID NO. 11). An example of this embodiment is one in which the nucleotide sequence of FVII is modified such that Pro-149 is changed to Arg-149 and Gly-151 is changed to Lys-151. The resulting amino acid sequence Arg-Gln-Lys-Arg (SEQ ID NO. 10) is a signal for precursor cleavage by the protease furin. Other examples for producing a furin cleavage site in the nucleotide sequence of FVII include substituting amino acids 147 through 150, 148 through 151, 150 through 153 or amino acids 151 through 154 with suitable amino acids to produce a furin cleavage site with the sequence Arg-X-Lys-Arg (SEQ ID NO. 17) or Arg-X-Arg-Arg (SEQ ID NO. 18).

4) In the "Examples" section on page 24 of the instant specification, please replace the paragraph that starts with the following sentence, "The factor VII cDNA was cloned by PCR (Perkin Elmer, 25 cycles) from a human liver..." with the following amended paragraph:

> The factor VII cDNA was cloned by PCR (Perkin Elmer, 25 cycles) from a human liver cDNA library (Clontech) using primer 5432JS (5' - CTAGCCTAGG CCACCATGGTCTCCCAGGCC CTCAGGCTC -3') (SEQ ID NO. 12) and primer 5433JS (5' - CCTTAATTAA CTAGGGAAAT GGGGCTCGCA GGAG -3') (SEQ ID NO. 13). The PCR product was cloned into a pCR-Blunt-II TOPO vector (Invitrogen), sequenced for accuracy and then subcloned into pCMV expression vector, which has the CMV promoter/enhancer and an SV40 polyA.

5) In the "Examples" section on page 24 of the instant specification, please replace the paragraph that starts with the following sentence, "The factor VII cDNA was cloned by PCR from the plasmid pCMV/hFVII using..." with the following amended paragraph:

The factor VII cDNA was cloned by PCR from the plasmid pCMV/hFVII using primer 5432JS shown above and primer 5479JS (5 '- GCTAGCCTAT CGGCCTTGGG G -3') (SEQ ID NO. 14). This construct contains the FVII leader sequence and amino acids #1 (Ala) to #152 (Arg). The PCR product has been cloned into the pCR-Blunt-II TOPO vector, sequenced for accuracy and then subcloned into the pCMV expression vector.

6) In the "Examples" section on page 25 of the instant specification, please replace the paragraph that starts with the following sentence, "The FVII heavy chain was cloned by three primer PCR from the plasmid pCMV/hFVII." with the following amended paragraph:

The FVII heavy chain was cloned by three primer PCR from the plasmid pCMV/hFVII. The three primers used were 5432JS, 5433JS and primer 5480JS (5 '- TGCACCGGCG CCGGCGCATT GTGGGGGGCA AGGTGT -3') (SEQ ID NO. 15). This construct contains the FVII leader sequence followed by amino acids #153 (Ile) to #406 (Pro). The PCR product has been cloned into the pCR-Blunt-II TOPO vector, sequenced and then subcloned into the pCMV expression vector.

7) In the "Examples" section on page 25 of the instant specification, please replace the paragraph that starts with the following sentence, "The cleavage site for the conversion of FVII to FVIIa has been mutated to a furin recognition site..." with the following amended paragraph:

The cleavage site for the conversion of FVII to FVIIa has been mutated to a furin recognition site using three primer PCR mutagenesis method. The original amino acid sequence #149 (Pro) and #151 (Gly) has been changed to #149 (Arg) and #151 (Lys) to generate the furin recognition site Arg^{149}-Gln-Lys-Arg^{152} (SEQ ID NO. 10). The PCR product has been cloned into the pCR-Blunt-II TOPO vector, sequenced and then subcloned into the pCMV expression vector.

8) In the "Examples" section on page 26 of the instant specification, please replace the paragraph that starts with the following sentence, "Human FVII cDNA was PCR amplified from a human liver Quick Clone cDNA (Clontech)..." with the following amended paragraph:

Human FVII cDNA was PCR amplified from a human liver Quick Clone cDNA (Clontech) and cloned into pCMV, a plasmid containing the CMV promoter and SV40 polyA and pLSP, a plasmid containing the AAT promoter and BGH polyA. The endogenous FVII cleavage site was mutated

to contain a furin recognition site using the primer (5 '- AGC AAA CGC CAA AAG CGA ATT GTG GGG GGC AAG -3') (SEQ ID NO. 16) which mutates Pro149 to an Arg and Gly151 to a Lys.

VIII. Conclusion

No fee is deemed necessary in connection with the filing of this communication. However, if any fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 07-1074.

Respectfully submitted,

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